Peer Review
Experimental Toxicology Division
N H E E R L

WATER

Analytical Aspects of Dose-Response Relations for Arsenicals

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Introduction

There is considerable uncertainty concerning the nature of its dose-response relationship for arsenic, a human carcinogen. The utility of population-based studies of the health effects of chronic exposure to arsenic in risk assessment is often limited by the lack of good measures of exposure. In the absence of data on individual exposure, researchers often use surrogate measures of exposure such as the concentration of arsenic in drinking water. Estimation of exposure from a surrogate can be misleading if there are substantial variations in intake among individuals or if there are other unquantified sources of exposure. In addition, because it is likely that many of the adverse health effects of arsenic are related to the production of methylated metabolites which contain either trivalent or pentavalent arsenic, it may be necessary to quantify the levels of these metabolites in biological samples.

Thus, two general issues can be defined concerning the analytical chemistry of arsenic.

- Can inorganic arsenic and its metabolites be accurately quantified in biological samples so that they can be used as biomarkers of exposure?
- What is the nature of the dose-response relation for inorganic arsenic and its metabolites in biological samples (e.g., urine, blood, nails)?

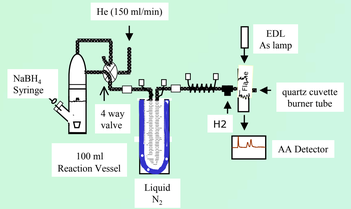
Methods

This laboratory has focused on the development and refinement of analytical procedures for the measurement of arsenicals in biological materials. We are particularly interested in the development of new methods for the differential analysis of methylated arsenicals that contain arsenic in the trivalent or pentavalent oxidation state.

The following methods are used in this laboratory.

- Determination of total arsenic in biological samples by hydride generation-atomic fluorescence spectrometry.
- Determination of speciated arsenicals in biological samples by reversed phase chromatography coupled with hydride generation-atomic fluorescence spectrometry.
- Determination of speciated arsenicals in biological samples by hydride generation-atomic absorption spectrometry. This method has been adapted for permit the selective detection of arsenicals containing pentavalent or trivalent arsenic.

A



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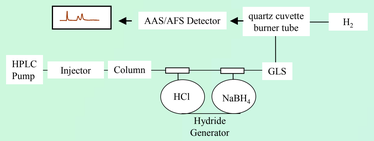
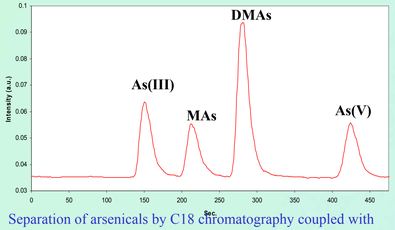


Apparatus for A) hydride generation-atomic absorption spectrophotometric speciation of arsenicals and B) for chromatographic separation coupled with hydride generation-atomic absorption or atomic fluoresence spectrophotometric speciation of arsenicals

Results

1

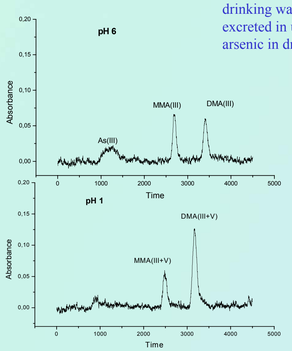


Separation of arsenicals by C18 chromatography coupled with detection by hydride generation-atomic absorption spectrophotometric speciation

2 Determination of the oxidation state of arsenicals by selective hydride generation

Example - Lysates from primary human hepatocytes cultured in medium containing 0.1 μM iAs^{III} for 24 hours were analyzed for contents of iAs^{III}, iAs^{V}, MAs^{III}, MAs^{V}, $DMAs^{III}$, and $DMAs^{V}$. Lysates processed for hydride generation at pH6 which allows formation of hydrides from arsenicals containing trivalent arsenic and for hydride generation at pH1 which allows formation of hydrides from arsenicals containing pentavalent or trivalent arsenic. Calculate amounts of each arsenical species containing pentavalent arsenic by the difference between the amount detected at pH 1 and the amount detected at pH 6.

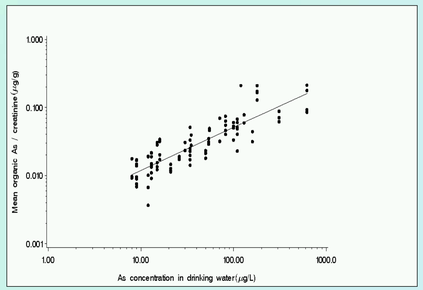
For the analyses shown here, the amount of each arsenical species has been determined.



iAs^{V}	iAs^{III}	MAs^{V}	MAs^{III}	$DMAs^{V}$	$DMAs^{III}$
ND*	0.9	ND	0.9	2.2	2.4

* ND = not detected; results in ng of As

3



Correlation between the concentration of inorganic arsenic in drinking water and the amount of methyl and dimethyl arsenic excreted in urine in a population chronically exposed to inorganic arsenic in drinking water

Conclusions

- Methods based on chromatographic separation are sufficient to separate inorganic arsenic from its major metabolites, methyl arsenic and dimethyl arsenic.
- Hydride generation which converts arsenicals into their corresponding arsines can be used in static and flow systems.
- Both atomic absorption and atomic fluorescence spectrometry can be used for the detection and quantitation of arsines.
- Selective generation of arsines can be used to determine the oxidation state of arsenic (III or V) in arsenicals.

Impact

- Refinement of analytical methods has assisted in design and conduct of population-based studies of health effects of exposure to inorganic arsenic.
- These studies using urinary arsenic as a biomarker provide new insight into dose-response relationships.
- New methods determine of the oxidation state of arsenic will support mode of action studies.

Future Directions

- Refine analytical methods to improve utility of urine, blood, and nails as biomarkers of exposure to arsenic.
- Test new separatory methods to improve ease and reliability of analyses.
- Refine detector design to improve sensitivity of analysis.
- Apply techniques to identify arsenic-binding that may play critical roles in the toxicity and carcinogenicity of arsenic.

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